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L6: Entry 4 of 13 File: USPT Apr 1, 1997

US-PAT-NO: 5616483
DOCUMENT-IDENTIFIER: US 5616483 A

TITLE: Genomic DNA sequences encoding human BSSL/CEL

DATE-ISSUED: April 1, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bjursell; Karl G.	Partille			SE
Carlsson; Peter N. I.	Goteborg			SE
Enerback; Curt S. M.	Molndal			SE
Hansson; Stig L.	Ume.ang.			SE
Lidberg; Ulf F. P.	Goteborg			SE
Nilsson; Jeanette A.	Goteborg			SE
Tornell; Jan B. F.	Vastra Frolunda			SE

US-CL-CURRENT: 435/198; 435/320.1, 435/325, 435/353, 435/354, 536/23.2

CLAIMS:

We claim:

1. An isolated genomic DNA molecule encoding for biologically functional human bile salt stimulated lipase/carboxylic ester lipase (BSSL/CEL).

2. The DNA molecule according to claim 1 which is shown in SEQ ID No: 1 in the Sequence Listing.

3. A replicable expression vector which carries and is capable of mediating the expression of a DNA molecule according to either one of claims 1-2, encoding human BSSL/CEL.

4. A vector according to claim 3 capable of encoding biologically functional BSSL/CEL and containing regulatory elements of genes selected from the group consisting of whey protein genes and casein genes, which directs expression of BSSL/CEL in the mammary gland of a non-human mammal.

5. A vector according to claim 3 which is the vector pS452 (DSM 7499).

6. A cell derived from a multicellular organism and harboring a vector according to claim 3.

7. A process for production of human BSSL/CEL, comprising (a) inserting a DNA molecule as defined in either one of claims 1-2 in a vector which is able to replicate in a specific host cell; (b) introducing the resulting recombinant vector into a host cell; (c) growing the resulting cell in or on a culture medium for expression of the polypeptide; and (d) recovering the polypeptide.

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DATE: Wednesday, February 12, 2003 Printable Copy Create Case

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L2	(oxidas$3 same (promot$3 same (methano$3 same pastori$3 same (vecto$3 or plasmi$3))))	91	L2
L1	(oxidas$3 same (promot$3 same (methano$3 same pastori$3 same (vecto$3 or plasmi$3))))	91	L1

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L5: Entry 1 of 2 File: USPT Oct 27, 1998

US-PAT-NO: 5827683
DOCUMENT-IDENTIFIER: US 5827683 A

TITLE: Nucleic acids encoding BSSL variants

DATE-ISSUED: October 27, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Blackberg; Lars Gustav	Ume.ang.			SE
Edlund; Michael	Ume.ang.			SE
Hansson; Stig Lennart	Ume.ang.			SE
Hernell; Olle Carl Edward	Ume.ang.			SE
Lundberg; Lennart Gustav	Billdal			SE
Stromqvist; Mats Olof	Ume.ang.			SE
Tornell; Jan Birger Fredrik	Vastra Frolunda			SE

US-CL-CURRENT: 435/69.1; 435/200, 435/243, 435/320.1, 435/325, 435/69.7, 435/70.1, 435/70.3, 435/71.1, 536/23.1, 536/23.2, 536/23.5

CLAIMS:

We claim:

1. A nucleic acid encoding a variant BSSL polypeptide that retains Bile Salt Stimulated Lipase activity, wherein one or more, but not all, of the amino acids in the region corresponding to amino acids 536-722, inclusive, of SEQ ID NO: 3 have been deleted.

2. The nucleic acid according to claim 1, wherein said variant BSSL polypeptide has a phenylalanine at its c-terminus.

3. The nucleic acid according to claim 1, wherein said variant BSSL polypeptide comprises the sequence Gln-Met-Pro within 50 amino acids of its C-terminus.

4. The nucleic acid according to claim 1, wherein said variant BSSL polypeptide comprises the sequence shown as residues 712-722 of SEQ ID NO: 3 within 50 amino acids of its C-terminus.

5. The nucleic acid according to claim 1, wherein said variant BSSL polypeptide comprises fewer than 16 repeat units, said repeat units being those of 33 nucleotides each, designated as such in SEQ ID NO: 1 in the Sequence Listing.

6. The nucleic acid according to claim 11, wherein said variant BSSL polypeptide comprises the amino acid sequence of SEQ ID NO: 5, 6, or 9.

7. A nucleic acid encoding a variant BSSL polypeptide that retains Bile Salt Stimulated Lipase activity, wherein the amino acid sequence of the polypeptide is that shown as SEQ ID NO: 3 in the Sequence Listing except that the nucleic acid encodes for an amino acid other than asparagine at position 187 of the polypeptide.

8. A nucleic acid according to claim 7, wherein the variant BSSL polypeptide comprises the amino acid sequence of SEQ ID NO: 7 in the Sequence Listing.

9. A hybrid gene comprising a nucleic acid according to claim 1 or 2 operably linked to a sequence that mediates expression of said hybrid gene in a cell of interest.

10. A recombinant expression vector comprising a hybrid gene according to claim 9.

11. A recombinant expression vector according to claim 10, wherein said vector is the bovine papilloma virus vector pS258, pS259 or pS299.

12. A recombinant cell comprising the vector of claim 10.

13. A recombinant cell according to claim 12, wherein said cell is selected from the group consisting of the murine cell line C127 and E coli.

14. A method of producing a BSSL protein having Bile Salt stimulated Lipase activity comprising:

(a) growing the recombinant cell according to claim 12 under conditions suitable for expression of said protein; and

(b) recovering said protein from said cell.

15. The method or claim 14 wherein said recombinant expression vector is the bovine papilloma virus vector pS258, pS259 or pS299.

1: Gene 1996 Oct 24;177(1-2):163-7 Related Articles

Inducible expression of a heterologous protein in Hansenula polymorpha using the alcohol oxidase 1 promoter of Pichia past

Raschke WC, Neiditch BR, Hendricks M, Cregg JM.

Salk Institute Biotechnology/Industrial Associates, Inc. (SIBIA), La Jolla, CA 9 USA.

Pichia pastoris (Pp) and Hansenula polymorpha (Hp) are methylotrophic yeasts commonly used for industrial purposes. Growth of either of these yeasts in the presence of methanol as the carbon source results in high-level induction of alc oxidase expression. The respective alcohol oxidase genes, AOX1 in Pp and MC Hp, have similar regulatory characteristics. Our studies show that the Pp AOX1 promoter (AOX1p) can be used for methanol-induced expression of a heterolog gene in Hp. Furthermore, the size of an AOX1p-heterologous gene-AOX1 termi cassette transcript synthesized in Hp is indistinguishable from that synthesized i suggesting that transcription both initiates and terminates at the same sites in bo yeast species. Induction of AOX1p in Hp demonstrates that the methanol-induc regulatory mechanism in Hp is able to recognize and activate the Pp promoter ir of extensive sequence variations between AOX1p and MOXp.

PMID: 8921862 [PubMed - indexed for MEDLINE]

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L6: Entry 2 of 13 File: USPT Jun 9, 1998

US-PAT-NO: 5763739
DOCUMENT-IDENTIFIER: US 5763739 A

TITLE: Transgenic non-human mammals producing BSSL variants

DATE-ISSUED: June 9, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Blackberg; Lars Gustav	Ume.ang.			SE
Edlund; Michael	Ume.ang.			SE
Hansson; Stig Lennart	Ume.ang.			SE
Hernell; Olle Carl Edward	Ume.ang.			SE
Lundberg; Lennart Gustav	Billdal			SE
Stromqvist; Mats Olof	Ume.ang.			SE
Tornell; Jan Birger Fredrik	Vastra Frolunda			SE

US-CL-CURRENT: 800/18; 435/69.1, 800/14, 800/15, 800/16, 800/17, 800/21, 800/25, 800/7

CLAIMS:

We claim:

1. A process of producing a transgenic non-human female mammal that produces in mammary recoverable amounts of a human BSSL variant that retains BSSL activity, comprising

(a) introducing an expression system into a fertilized egg of a non-human mammal, wherein

(i) said expression system comprises a hybrid gene that is expressed in the mammary gland of an adult female mammal harboring said hybrid gene in its genome,

(ii) said hybrid gene comprises a DNA molecule encoding a human BSSL variant that retains BSSL activity and operatively linked to a promoter of a gene that is expressed in the mammary gland of a mammal, and wherein the DNA molecule encodes a BSSL variant in which one or more but not all of the amino acids in the region corresponding to amino acids 536-722, inclusive, of SEQ ID NO:3 have been deleted;

(b) introducing the fertilized egg containing the expression system into a host non-human mammal of the same species as the fertilized egg;

(c) allowing the host non-human mammal to produce progeny; and

(d) selecting a female progeny non-human mammal that produces recoverable amounts of the BSSL variant in its milk.

2. A process of producing a transgenic female mouse that produces in mammary tissue recoverable amounts of a human BSSL variant that retains BSSL activity and is substantially incapable of expressing a BSSL gene of the mouse itself,

comprising

(a) destroying the native BSSL gene-expressing capability of the mouse by mutation of mouse DNA sequences responsible for the expression of the native BSSL genes so that substantially no murine BSSL is produced, and injecting an expression system according to claim 1 or 6 into a fertilized egg or embryo cell of said mouse, or

(b) destroying the native BSSL gene-expressing capability of the mouse by replacing all or part of the BSSL genes of cells of the mouse by homologous recombination with an expression system according to claim 11 or 6, and introducing the genetically modified cells into a developing embryo,

and then transferring the genetically modified egg or embryo produced by (a) or (b) above into a pseudopregnant female mouse to develop into a transgenic female mouse having the BSSL expression system in its germline and producing in its mammary tissue recoverable amounts of a human BSSL variant that retains BSSL activity.

3. A transgenic non-human female mammal produced by the process of claim 1 or 6, wherein said mammal produces in mammary tissue recoverable amounts of a human BSSL variant that retains BSSL activity.

4. A transgenic non-human female mammal according to claim 3, wherein said mammal produces in mammary tissue recoverable amounts of a human BSSL variant that retains BSSL activity, which mammal is selected from the group consisting of mice, rats, rabbits, sheep, pigs, and cattle.

5. Progeny of a transgenic non-human mammal according to claim 3, wherein said progeny produce in mammary tissue recoverable amounts of a human BSSL variant that retains BSSL activity.

6. A process of producing a transgenic non-human female mammal that produces in mammary recoverable amounts of a human BSSL variant that retains BSSL activity, comprising

(a) introducing an expression system into a fertilized egg of a non-human mammal, wherein

(i) said expression system comprises a hybrid gene that is expressed in the mammary gland of an adult female mammal harbouring said hybrid gene in its genome,

(ii) said hybrid gene comprises a DNA molecule encoding a human BSSL variant that retains BSSL activity and operatively linked to a promoter of a gene that is expressed in the mammary gland of a mammal and wherein the DNA molecule encodes a BSSL variant whose amino acid sequence is at least 90% identical to the amino acid sequence shown as SEQ ID NO:7 in the Sequence Listing and does not encode for asparagine at position 187 of the variant;

(b) introducing the fertilized egg containing the expression system into a host non-human mammal of the same species as the fertilized egg;

(c) allowing the host non-human mammal to produce progeny; and

(d) selecting a female progeny non-human mammal that produces recoverable amounts of the BSSL variant in its milk.

7. A transgenic female mouse produced by the method of claim 2 that produces in mammary tissue recoverable amounts of a human BSSL variant that retains BSSL activity, and is substantially incapable of expressing a BSSL gene of the mouse itself.

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L3          292 DUP REM L2 (13 DUPLICATES REMOVED)
L4           12 S PASTORI? (S) METHAN? (S) HUMA? (S) (LIPAS? OR ESTERAS?)
L5            4 DUP REM L4 (8 DUPLICATES REMOVED)
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